

IN THE CLAIMS:

Claim 1 (currently amended) A recombinant nucleotide sequence of expression cassette OXY-1 of SEQ ID No. 1.

Claim 2 (currently amended) A ~~modified~~ recombinant staphylokinase SAK-2 gene of SEQ ID No. 2.

Claim 3 (currently amended/withdrawn) A peptide sequence of ~~modified~~ recombinant staphylokinase SAK-2 gene[[,]] of SEQ ID No. 3.

Claim 4 (currently amended) A plasmid pRM1 contained in *E. coli* having International Deposition No. BPL-0019.

Claim 5 (currently amended) A plasmid pOXYSAK-1 contained in *E. coli* having International Deposition No. BPL-0020.

Claim 6 (currently amended) A plasmid pOXYSAK-2 contained in *E. coli* having International Deposition No. BPL-0021.

Claim 7 (original) A recombinant *E. Coli* of International Deposition No. 5146, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, having a plasmid pRM1 of International Deposition No. BPL-0019.

Claim 8 (original) A recombinant *E. Coli* of International Deposition No. 5147, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, having a plasmid pOXYSAK-1 of International Deposition No. BPL-0020.

Claim 9 (original) A recombinant *E. Coli* of International Deposition No. 5148, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, having a plasmid pOXYSAK-2 of International Deposition No. BPL-0021.

Claim 10 (currently amended/withdrawn) A process for over-producing staphylokinase and its analogues by modulating level of oxygen ~~level of its growth medium in a host system~~, said method comprising the steps of:

- ~~a. preparing a piece of DNA carrying genetic information for the production of staphylokinase,~~
- ~~b. modifying 10 amino terminal residues of SAK encoding DNA, wherein Lys6 and Lys8 residues of SAK are changed to small neutral amino acid residues,~~
- ~~c. constructing DNA expression cassette OXY-1,~~
- ~~d. integrating piece of DNA obtained at step (a) or step (b) with the OXY-1 to obtain pOXYPRO,~~
- a. isolating a staphylokinase gene encoding an SAK protein,
- b. modifying codons in the gene encoding Lys6 and Lys8 residues present at an amino-terminal end of the SAK protein to obtain a

modified DNA,

c. providing the recombinant nucleotide sequence of expression

cassette OXY-1 as claimed in claim 1,

d. integrating the modified DNA obtained in step (b) with the
recombinant nucleotide sequence of expression cassette OXY-1 to
obtain a product comprising pOXYPRO,

e. ~~transferring integrated product of step (d) on a plasmid vector~~
integrating the product obtained in step d with plasmid vector PRM
1 to obtain plasmid construct constructs pOXYSK-1, and
pOXYSK-2 respectively,

f. introducing the plasmid constructs of step (e) into a host systems system,

g. culturing the host cell for over-production of SK or its derivatives under
high aeration and changing level of oxygen below 5% of atmospheric
oxygen level when cell growth reaches to exponential phase to obtain
cell mass,

h. lysing the of the cell mass cells of step (g) to separating separate cell
lysate from the cellular debris, and thereby obtaining the
staphylokinase and its analogues.

Claim 11 (currently amended/withdrawn) A process as claimed in claim 10, wherein
the Lys6 and Lys8 residues of the SK protein are changed into small and neutral
amino acid residues.

Claim 12 (withdrawn) A process as claimed in claim 10, wherein the plasmid vector is a high or medium copy number plasmid.

Claim 13 (currently amended/withdrawn) A process as claimed in claim 10, wherein the host system is selected from a the group ~~comprising~~ consisting of *E. coli*, *Bacillus*, and Yeast.

Claim 14 (withdrawn) A process as claimed in claim 10, wherein the sequence of OXY-1 is modified depending upon the host system.

Claim 15 (currently amended/withdrawn) A process as claimed in claim ~~11~~ 10, wherein the amino acids acid residues are selected from a the group consisting of ~~comprising~~ Alanine[~~],~~] and Glycine.

Claim 16 (currently amended/withdrawn) A process as claimed in claim 10, wherein the culturing is in growth medium is comprising Luria Broth (LB) medium.

Claim 17 (withdrawn) A process as claimed in claim 10, wherein culturing the host cell for over-production of SAK or its derivatives at shake flask culture or at fermentation.

Claim 18 (currently amended/withdrawn) A process as claimed in claim 17, ~~wherein~~ comprising culturing the host cell till ~~O.D.₆₀₀~~ O.D.₆₀₀ reaches 0.6 to 0.7.

Claim 19 (currently amended) A process as claimed in claim 17, wherein the fermentation is a two-stage fed-batch fermentation.

Claim 20 (currently amended/withdrawn) A process as claimed in claim 10, wherein comprising obtaining the cell mass by centrifugation or filtration.

Claim 21 (currently amended/withdrawn) A process as claimed in claim 10, wherein the lysing of the cells is by a method selected from a the group consisting of ~~comprising~~ sonication, chemical, and mechanics lysis.

Claim 22 (currently amended/withdrawn) A process as claimed in claim 10, wherein comprising separating the cell lysate from the cellular debris by centrifugation.

Claim 23 (withdrawn) A method of dissolving blood clot in a subject in need thereof, said method comprising step of administering pharmaceutically effective amount of streptokinase analogue SAK-2, optionally along with additive(s).

Claim 24 (withdrawn) A method as claimed in claim 23, wherein the additive is selected from a group comprising nutrients consisting of proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste, and/or pharmaceutically acceptable carrier, excipient, diluent, or solvent.

Claim 25 (withdrawn) A method as claimed in claim 23, wherein the SAK-2 and additives are in a ratio ranging between 1:10 to 10:1.